FORM-REGULATION IN CERIANTHUS, VIII.

Supplementary Partial Discs and Heteromorphic Tentacles.

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The Regeneration of Supplementary Partial Discs and Correlated Regulation.

The regeneration of supplementary partial discs and tentacles in *Cerianthus* from the lateral body-wall was first described by Loeb ('91). The method of procedure employed in order to produce results of this kind was partial transverse section of the body-wall at one or more levels. According to Loeb it is necessary to delay the healing of the wound for a few days in order to bring about tentacle regeneration in connection with it. The regeneration of tentacles and disc always occurs in connection with the lower (aboral) side of the wound. The number of tentacles borne by the supplementary disc is a fraction of the whole number characteristic of the complete disc and varies with the portion of the circumference involved in the cut. When the cut occurs near the oral end of the body the supplementary disc possesses a mouth, but discs regenerated at a lower level, *i. e.*, further aborally, possess no mouth.

Loeb regarded these experiments as affording an important indication of the relation of growth to turgor of the cells. After a transverse cut is made in one side of the body-wall the tentacles oral to this region "lose their turgor" while the other tentacles retain theirs. This fact, as Loeb believes, indicates that the distension or erection of the tentacles cannot be due to water under pressure in the enteric cavity since if that were the case an opening into the enteron would cause the collapse of all tentacles, and he concludes that the cut has in some way reduced the turgor of the cells, comparing the effect with the withering of plant tissues after the stem is cut. No suggestion as to the manner in which the cut effects loss of turgor in the cells of the tentacles is given. Certainly the case is not comparable with that of the plant for the tissues of *Cerianthus* are bathed internally and externally by water and it is very difficult to see how or why a cut on one side of the body at a distance of two to three centimeters from the tentacles should cause loss of turgor in those tentacles directly above it. There are no vessels or tubes of any kind running longitudinally in the body-wall. If the shock or irritation resulting from the cut is the cause of the loss of turgor why should it be transmitted only in the oral direction?

Apparently Loeb still holds the view originally expressed regarding these phenomena for he has recently called attention to these experiments and to his conclusions (Loeb, '03). As a matter of fact, however, the regeneration of these supplementary partial discs and tentacles and the reduction of the tentacles oral to them constitute most striking evidence in favor of the view that internal, *i. e.*, enteric, water-pressure is an important regulative factor in *Cerianthus*. There is not the slightest ground in support of the conclusion that the direct effect of a cut upon intracellular turgor extends beyond the cells in the immediate vicinity of the injury.

In my own experiments both *C. membranaceus* and *C. solitarius* were used, the latter species chiefly. Similar results were obtained from both species, as in the case of other regulative phenomena. Description of a few of my experiments and a brief analysis of the results obtained will show very clearly that the supplementary partial discs and tentacles regenerate in exactly the same manner as typical localized structures, and that reduction of tentacles above the accessory discs is due, like other cases of tentacle-reduction described, to decrease in internal pressure.

The experiments group themselves under several categories: the results differ somewhat according as the operation is within the œsophageal region or aboral to it; moreover, the effects of these operations both upon regeneration of tentacles and upon their reduction must be considered. I shall discuss first the regulation of lateral openings in the œsophageal region in relation to tentacle regeneration, and tentacle reduction, and then the regulation of lateral openings in regions of the body aboral to the œsophagus in relation to tentacle-regeneration and reduction.

Supplementary Discs in the Œsophageal Region.

The method employed in producing partial supplementary discs in the œsophageal region was as follows: a transverse cut was made in one sideof the body-wall near the middle of the œsophageal region (Fig. 1), thus interrupting on one side of the body the continuity of both œsophagus and body-wall, while on the opposite side both remained intact. Loeb removed small pieces of the body-wall near the middle of the œsophageal region in his experiments of this kind, thus preventing rapid healing of the cut edges (Loeb, '91, text fig. 1). I found, however, that this was not necessary in the œsophageal region, the mere transverse slit being in most cases sufficient to bring about the desired results. Neither was it necessary to delay or prevent the union of the margins of the body-wall by artificial means such as the wire netting employed by Loeb for this purpose.[1]

In my experiments the pieces were usually left undisturbed after the operation, though occasionally it was necessary to cut a piece a second time in order to cause the production of a supplementary disc. Loeb ('91, p. 56) himself noted that the cut showed a much stronger tendency to remain open when situated near the oral end, but apparently was unaware that this was due to the presence of the œsophagus.

The history of tentacle regeneration in a piece of this kind is given as an example.

Series 42.

October 24, 1902. — A large specimen (*C. solitarius*) of normal appearance was subjected to the operation indicated in Fig. 1. First disc and tentacles were removed by a transverse cut just aboral to the disc. Then a transverse cut extending about half way through both body and œsophagus was made near the middle of the œsophageal region. After section the piece collapsed, as in all cases where the enteron is opened, and became distended only when closure of the enteron occurred, either by union of cut surfaces or by approximation.

[1] The meshes of this netting were somewhat smaller than the diameter of the body and the animals, being laid upon the netting, pushed their bodies through it, aboral ends first. until the level of the partial transverse cut was reached. The wires being forced into the cut prevented further movement and also, according to Loeb, delayed union of the two cut surfaces.

November 1. — Eight days after section. On the oral end of the piece the œsophagus and body-wall have united about the whole circumference of the body, the usual result in such cases; the free surfaces of the body-wall formed by the transverse cut have not united with each other, but each has united with the corresponding cut œsophageal surface. Fig. 2,[1] a diagrammatic longitudinal section of the body at this stage, shows what has occurred more clearly than is possible by description. It is evident that that part of the body directly over (*i. e.*, oral to) the lateral cut has closed in such a manner that its enteric cavity

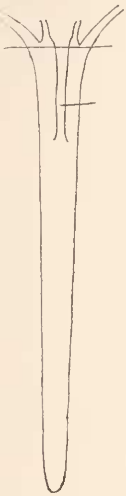

FIG. 1.

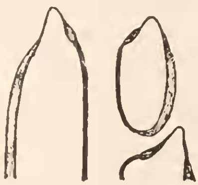

FIG. 2.

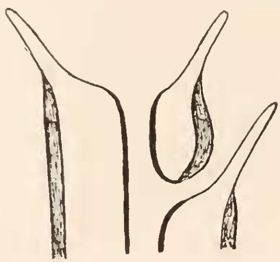

FIG. 3.

is not in communication with that of other parts of the body nor with the exterior. Orally and aborally it is closed by the union of œsophagus and body-wall; each intermesenterial chamber is separated from adjoining chambers by the intervening mesentery. Thus, as regards this region, conditions are similar to those ex-

[1] The different regions are distinguished in these figures in a somewhat conventional manner. The old body-wall is represented by two heavy lines (ectoderm and entoderm) with fine longitudinal lines indicating the longitudinal muscles between them. The œsophageal region is represented by a very heavy single line and regenerated regions by a much lighter single line. In Figs. 7 and 8 portions of old disc and tentacles are represented by a double line and regenerated disc and tentacles by a single line.

isting in the œsophageal pieces described in a previous paper (Child, '04*d*). Consequently water can enter this portion only by diffusion or as a secretion.

The lower (*i. e.*, aboral) margin of the transverse cut and the oral end of the body on the uncut side present similar conditions (Fig. 2). In both regions the union of œsophagus and body-wall produces conditions apparently favorable for typical tentacle regeneration; in both the intermesenterial chambers are in free communication with the main enteric cavity and distension and free movement of the circulatory currents is therefore possible.

It is clear from the diagram (Fig. 2) that each of the discs will form a mouth. The relations of these two mouths is also sufficiently evident from the figure.

If the conclusions reached in preceding papers ('04*b*, '04*c*, '04*d*) are valid we may expect that tentacle-regeneration will occur in the typical manner on the oral margins of the body of the uncut side and on the aboral margin of the transverse cut, *i. e.*, the lower margin which corresponds to an oral end with respect to that part of the body aboral to it.

On the oral end of the body over the transverse cut regeneration must be delayed if internal water-pressure is effective, since only the slight distension due to diffusion or secretion is possible in this region. The history of the piece fulfills all expectations.

For convenience the regenerating region at the oral end of the piece may be designated as terminal and that on the side of the body as lateral. It is also necessary to distinguish between the cut side of the body, that on which the lateral cut is made and the uncut side.

Regeneration of the marginal tentacles has already begun in this piece (Fig. 2); the terminal marginal tentacles on the uncut side are 1 mm. in length, those on the cut side are minute buds 0.25–0.5 mm. in length. The lateral marginal tentacles are about 0.5 mm. in length. In appearance they are more advanced than the terminal tentacles on the cut side but somewhat less advanced than those on the uncut side. The portion of the body between the lateral cut and the oral end is very evidently less distended than the other parts.

November 6. — Thirteen days after section. Terminal marginal tentacles on uncut side 3 mm., on cut side 2–2.5 mm.; lateral marginal tentacles 3 mm. The regeneration of the tentacles over the lateral cut is somewhat retarded. Fig. 3 represents a diagrammatic longitudinal section through the œsophageal region at this stage.

November 12. — Nineteen days after section (Figs. 4 and 5). Terminal marginal tentacles on uncut side 6–7 mm.; on cut side 1 mm.: lateral marginal tentacles 6–7 mm. Terminal labial tentacles on uncut side 1 mm.; on cut side absent: lateral labial tentacles 1 mm. The terminal disc on the uncut side and the partial lateral disc are well distended, while that part of the terminal disc which lies over the cut is collapsed and wrinkled. The distended portion of the lateral disc and the terminal disc

Fig. 4.

are gradually approaching the same level. At this stage the regeneration of the terminal tentacles over the cut is not only retarded but has ceased and the tentacles are decreasing in size. The wrinkled appearance of the portion of the body bearing these tentacles shows clearly that it does not share in the distension of the other parts.

The diagrammatic figures (Figs. 4 and 5) represent respectively the relations of parts in longitudinal section and in oral aspect. In Fig. 5 the shrivelled half of the margin with its reduced tentacles is clearly shown. One tentacle at each end of this reduced region is somewhat longer than the other reduced tentacles. Evidently this tentacle on each side arises over the intermesenterial chamber abjoining the distended part of the body. Probably the greater length of these two as compared with the other reduced tentacles is due to the greater degree of distension of the intermesenterial chambers below them. It may be that these chambers are not completely shut off from the enteric cavity or possibly the pressure of the water in the distended region causes filtration of water into these two chambers. Figs. 4 and 5 illustrate very clearly the relation between characteristic form and internal water pressure.

November 20. — Twenty seven days after section (Fig. 6).

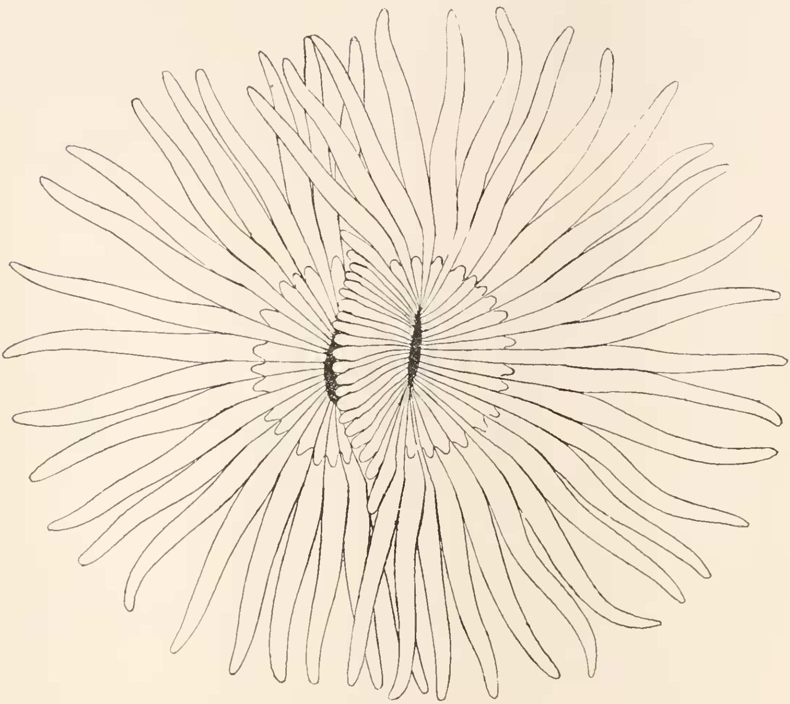

FIG. 5.

Terminal marginal tentacles on uncut side 8–9 mm.; on cut side barely visible 0.25 mm.: lateral marginal tentacles 8–9 mm. Terminal labial tentacles on uncut side 2 mm., on cut side absent: lateral labial tentacles 2 mm. The portion above the lateral cut is much shrivelled while all other parts are fully distended and in good condition. The lateral and terminal discs are now at almost the same level (Fig. 6), the part which originally formed one side of the terminal disc being now represented only by the narrow shrivelled strip separating the two discs. About thirty days after section the shrivelled portion separating the two discs was ruptured by the increase in diameter of the discs. Each portion of it remained attached to the disc on its own side but gradually underwent atrophy. Reference to Fig. 6 renders it evident that this shrivelled strip was all that separated the two mouths: with its rupture the two mouths became one. The two discs, or two parts of the disc continued to approach the same level, the constriction marking the region where the shrivelled strip stretched across (Fig. 5) gradually disappeared and on December 12, forty-nine days after section the

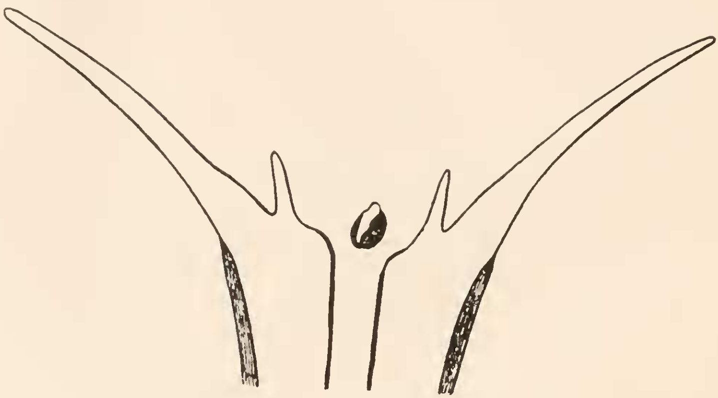

Fig. 6.

specimen was normal in appearance, with marginal tentacles about 12 mm. in length and labial tentacles about 3 mm.

The history of other specimens of the same sort corresponds closely with that of the piece just described. It is possible of course to modify the results in various ways: for example the lateral cut may be made more or less deep and thus cause the isolation of a greater or less number of intermesenterial chambers above it and consequently the retardation in regeneration and the later reduction of a larger or smaller number of tentacles. It is possible to make the cut so deep that only three or four tentacles remain distended on the terminal disc all the rest appearing on the lateral disc. On the other hand, if the cut involves only a small portion of the œsophageal circumference, only a few tentacles are retarded and correspondingly only a few appear on the lateral disc. If the cut is not deep enough to reach the œsophagus, but involves only the body-wall, the cut surfaces of the body-wall unite within a few days and regeneration of the terminal disc proceeds in the typical manner.

Turning now to the consideration of specimens with fully developed normal tentacles, the effect of a lateral cut in the œsophageal region is not difficult to understand if we recall the reduction and atrophy of tentacles in consequence of reduced internal pressure which was discussed in the preceding paper (Child, '04*e*), and if we bear in mind also the method of closure in series 42. For comparison with series 42 the history of a piece of this kind is given briefly.

Series 10.

September 12, 1902. — A specimen with marginal tentacles about 25 mm. and labial tentacles about 8–9 mm. in length was used. A lateral transverse cut extending about half way through the body was made in the œsophageal region as in Series 42 (Fig. 1) but in the present case the original disc and tentacles were not removed as in Series 42.

September 19. — Seven days after section. Closure has occurred as shown in Fig. 7 and the specimen is distended. The tentacles on the uncut side retain their original length ; marginal 25 mm., labial 8 mm. ; those on the cut side are reduced — marginals 15–18 mm., labials 6 mm. The portion above the lateral cut in which, as in Series 42, the intermesenterial chambers are completely shut off from the exterior and from the main enteric cavity, is less distended than the other portions as indicated in Fig. 7. In this figure only the bases of the old tentacles are shown but the smaller size of those on the right is indicated.

The cut surfaces of œsophagus and body-wall on the lower (aboral) side of the cut have united as in Series 42 and now the regenerating marginal tentacles are about 2 mm. in length on this supplementary disc (Fig. 7).

October 3. — Twenty-one days after section (Fig. 8). The tentacles on the uncut side still retain nearly their original length

— marginal 20–22 mm., labial 7–8 mm. The tentacles oral to the lateral cut are now greatly reduced — marginal 3–4 mm., labial 1–2 mm. — and the tips are shrivelled; the whole region above the lateral cut is greatly shrunken and wrinkled, containing almost no water (Fig. 8).

Aboral to the cut the supplementary tentacles have regenerated in the typical manner — marginals 10 mm., labials 3–4 mm. The new supplementary disc on the right, and the left half of the old disc now lie at nearly the same level.

A few days later the shrivelled strip bearing the reduced tentacles was ruptured and a part dropped off. The remaining portions underwent complete resorption within a few days, and a disc of typical form resulted. One half of this disc represented what remained of the old disc and its tentacles were the tentacles of

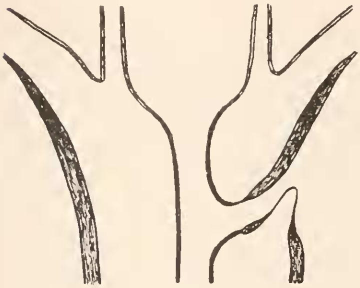

Fig. 7.

Fig. 8.

the original specimen; the other half was the regenerated supplementary disc. The tentacles on the old portion were still considerably longer than on the regenerated portion, but a gradual equalization in length occurred as might be expected since conditions of internal pressure are similar about the whole circumference.

The history of other similar specimens is similar in all essential respects to that described, though the number of tentacles which undergo atrophy vary according to the size of the lateral cut. It is possible to modify these experiments in many ways but in every case that part which has no communication with the main enteric cavity or the exterior and so does not remain distended undergoes atrophy, while those regions in which internal pressure persists either retain their form and size or regenerate as the case may be.

The atrophy of the tentacles above the cut in series 10 is very clearly due to the same factor which caused retardation in regeneration and later reduction and atrophy of the tentacles over the cut in series 42. In both cases the conditions in the strip above the lateral cut are exactly similar to those which exist in œsophageal pieces (Child, '04*d*). Comparison of the history of these strips with that of the œsophageal pieces will show that both are affected in exactly the same way. Some degree of internal pressure may be established by osmosis or secretion after closure, but this gradually diminishes, the tentacles atrophy, and the whole piece shrivels and finally breaks up.

The relative change in level of the two partial discs, which results in the production of a disc of typical form and in typical position with respect to the body axes, is a features of special interest. It is impossible to determine from the appearance after regulation is completed whether the lateral supplementary disc moves orally to the level of the old disc, or *vice versa*, or finally whether both change their level, the old partial disc moving aborally and the new orally until they attain approximately the same level.

There is always in a regenerating end a certain amount of growth in the oral direction due undoubtedly to internal pressure. The supplementary partial disc changes its level in the oral direction in consequence of growth. This change brings it nearer to the level of the old disc, which of course has not been altered since its tissues are fully differentiated and not in a condition for rapid growth. Probably this oral movement of the regenerating disc is sufficient, when it is situated in the œsophageal region, to bring it approximately to the same level as the old disc. If differences in level remain after the regenerative growth is completed, I think it is probable that further regulation may occur in the manner discussed in connection with oblique pieces (Child, '04*d*), viz., through the attempt of the animal to orient disc and body-axis in a definite manner. In the pieces with supplementary partial discs in the œsophageal region the difference of level between the supplementary disc and the old disc is greater during the contracted condition than during distension, as in pieces with oblique discs. This fact indicates that the muscles of the body-wall below the supplementary disc are more completely relaxed during distension and orientation than those of the opposite side (Child, '04*d*). The tissues gradually adapt themselves to the altered conditions and the final result is the form in which orientation in the typical manner is possible, *i. e.*, the typical or normal form.

Supplementary Partial Discs Aboral to the Œsophagus.

Loeb ('91) found it possible to produce the partial discs at any level of the body except the extreme aboral region, but stated that the tendency to closure of the cut without the formation of a new disc is greater in the middle region than near the oral end. Moreover, he found that the partial discs produced in the middle region were without mouth-openings.

My results agree with Loeb's in regard to these points, but, since he did not turn his attention to the internal anatomy nor investigate the nature of the distension, the reasons for these differences did not become apparent to him. Apparently he was not even aware that in making the lateral cut near the oral end he was cutting into the œsophagus, while farther aborally only the general enteric cavity was opened. I desire, therefore, to point out certain very definite reasons why the results should differ according to the position of the lateral cut in the œsophageal region or aboral to it.

There are several points to be considered, but the question as to the effect of the lateral cut on the tentacles directly oral to it naturally takes precedence as one of the immediate consequences of the cut.

According to Loeb, a lateral cut in the middle or aboral region of the body does not cause any permanent difference between the tentacles directly oral to it and the others; here also my results agree with his. His Figs. 2 and 3 show cases of this kind in which the tentacles at the oral end are equal in length about the whole circumference.

If the collapse and reduction of tentacles be due to loss of intracellular turgor in consequence of the cut there is no reason apparent for the permanent reduction of the tentacles after a cut near the oral end and the absence of any such effect after a cut near the middle. The difference in the distance between cut and tentacles in the two cases is certainly not sufficient to justify the conclusion that in the one case a shock or stimulus of some sort reaches the tentacles while in the other case it does not, nor can it serve as a basis for explanation if the change be regarded as simply osmotic in nature, not as a reaction to a stimulus.

If, however, we consider the difference in the relations of parts in the two regions of the body and the effect upon the internal pressure all difficulty disappears. If the lateral cut be made at any of the levels indicated in Fig. 9 it will simply enter the intermesenterial divisions of the enteron which open axially into the central cavity everywhere aboral to the œsophagus, since the axial margins of the mesenteries hang free in the cavity. There is nothing with which either margin of the cut can unite except the other margin. When cuts at these levels close they can close only by means of union of the cut edges. It follows that the same relations between the part oral to the cut and the other regions of the body are not altered by the cut.

The immediate consequence of such a cut is loss of water from the enteron and complete collapse of body and all tentacles. Inrolling of the body-wall adjoining the cut soon begins and approximation of the inrolled portions is brought about by the elasticity of the other parts of the body-wall — especially that of the opposite side of the body (Child, '04*a*, Figs. 8 and 9). Occasionally when the cut is not far below the aboral end of the œsophagus, the contraction of the body-wall and of the thickened mesenterial margins and filaments in consequence of the cut almost or quite closes the intermesenterial chambers between the cut and the aboral end of the œsophagus. It follows that when water again enters the enteron through the œsophagus other regions will become distended, but these will not and consequently the corresponding tentacles will remain collapsed. At this stage the internal pressure is always much lower than that in normal animals since increase of the pressure beyond a certain point causes the approximated margins of the cut to separate and the water is lost. The point to which I wish to call attention, however, is this: when the lateral cut is only slightly aboral to the œsophagus the intermesenterial chambers oral to the cut may be closed temporarily and so the tentacles corresponding to them may remain less distended than the others as long as this condition continues. This difference is due simply to mechanical plugging of the openings of the intermesenterial chambers at the aboral end of the œsophagus and the consequent exclusion ot water. As soon as the cut is actually closed by new tissue the increasing distension causes stretching of the body-wall and mesenteries and the closure of the chambers can persist no longer. The tentacles in this region now become as fully distended as the others and since the period of their collapse is usually short in such cases, are soon equal in length to the others. If the intermesenterial chambers remain contracted and plugged during a long time considerable reduction of the corresponding tentacles may occur. Cases of this kind have occurred frequently among my specimens and it was always evident that the contracted condition of parts about the cut and especially oral to it was responsible for the absence of distension in tentacles oral to this region. If the collapse were osmotic in nature, as Loeb believes, there is no reason apparent why it should be permanent when the cut is in the œsophageal region and only temporary when the cut is a few millimeters lower.

Loeb found that lateral cuts in the middle and aboral regions showed a much greater tendency to close by union of their margins than those near the oral end. He does not attempt to account for this difference in any way, but the reason is evident at once when the part played by the œsophagus is understood. In the œsophageal region the cut margins of œsophagus and body-wall almost always come into contact in consequence of the inrolling (Fig. 2). The distension following union of the parts prevents the two cut surfaces of the body-wall from coming into contact with each other and, therefore, they never unite and an opening remains which is in reality a new mouth. If they happen to come into contact with each other instead of with œsophageal cut edges they unite but the usual result is determined by the fact that conditions are much more favorable for the union of body-wall and œsophagus than for body-wall and body-wall.

On the other hand, if the cut is below the œsophagus the only possible union is body-wall and body-wall (Fig. 10). If the animal is left undisturbed the cut will close in the typical manner, and as soon as distension occurs the relations of parts are once more normal in all essential respects (Fig. 11). Both Loeb and myself agree that under these circumstances a formation of a supplementary disc is not possible.

It now remains to consider how conditions are altered if the animal is inserted into a mesh of the netting (see above, p. 95),

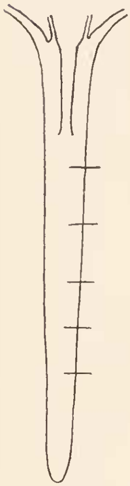

FIG. 9.

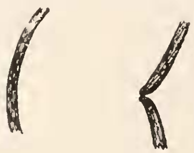

FIG. 10.

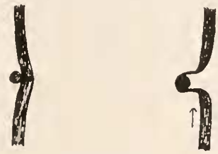

FIG. 13.

FIG. 11.

FIG. 14.

FIG. 12.

FIG. 15.

used by Loeb to cause the appearance of the supplementary disc. In Figs. 12, 13 and 14 the result of using the netting is shown. As long as the body is collapsed the netting has little effect (Fig. 12). Indeed, in my experience collapsed specimens react much less readily than others, and orientation in the netting rarely occurs until the animal is at least partly distended. It follows from this that the formation of supplementary discs after use of the netting is not necessarily due to delay in closure. As distension with water gradually occurs after closure the diameter of the body increases, but the wires of the netting prevent increase in diameter in the region of the cut. The result is indicated by Fig. 13. The thin, delicate new tissue is chiefly affected, being stretched in the direction of the tension, or more probably undergoing rapid growth in response to it. Thus a deep groove is formed on one side of the body and on the aboral side of this groove the new tentacles of the supplementary disc appear (Fig. 14).

Apparently the netting has merely prevented the body-wall from assuming the form shown in Fig. 11, or, in other words, it has prevented the obliteration of the angle in the body-wall due to inrolling, and has in fact made this angle less obtuse (cf. Figs. 12 and 13). If this conclusion be correct, and there is little room for doubt, it follows that the appearance of the supplementary disc is due, not to the presence of the cut, but to the bending inward of the body-wall in such a manner as to form in longitudinal section an angle opening aborally. But how can the formation of this angle determine this region for the formation of tentacles? The only answer to this question which seems to me at all satisfactory is the suggestion already made (Child, '04*b*, '04*c*, '04*d*) that the circulatory currents passing orally along the body-wall in each intermesenterial chamber strike against the inrolled body-wall and so produce areas of increased pressure to which the tissues react by growth and tentacles appear. The arrows in Figs. 11–14 indicate these currents. In no case within my experience have tentacles been seen to arise otherwise than on an inrolled portion of the body-wall. Ordinarily of course the inrolled region is a cut edge, but here the cut closed before the inrolling was brought about to any great extent.

If the formation of the angle in the body-wall instead of the presence of a cut surface is the essential condition for tentacle formation, it should be possible to bring about tentacle formation at any level capable of growth by constricting the body just oral to this level. I have attempted many times to carry out experiments of this kind by ligaturing the body at some level. All of these attempts have failed because the specimens parted in the plane of the ligature after a few hours if this was tied tightly, or crept out of it if it was at all loose. I am firmly convinced, however, that the production of these supplementary discs and tentacles at any level of the body capable of growth is possible without the presence of a cut surface, and I predict that as soon as a method is devised for retaining a sufficient local constriction at the same level for a few days without causing injury to the body-wall, the production of tentacles without relation to cut surfaces will be possible in *Cerianthus*.

Like the tentacles on the lower margin of an oblique piece (Child, '04*d*), the tentacles below a lateral cut when once growth has begun, continue to grow even though the animal is removed from the netting. By this time union of body-wall and mesenteries in the new relations is sufficiently advanced to cause the persistence for a time of the fold formed by the netting. In most cases, however, the fold is gradually obliterated by the internal pressure and the supplementary disc protrudes from the side of the body (Fig. 15).

Loeb called attention to the fact that only those supplementary discs which are situated near the oral end ol the body possess mouths, the others consisting merely of a partial ring of tentacles arising from a small surface resembling part of a disc but completely closed. Comparison of Figs. 2, 3, 4, 6, 7, 8 with Fig. 15 will show the difference in character of the supplementary structures at different levels. It is evident at once that the presence of the œsophagus is responsible for the formation of the new mouth in supplementary discs near the oral end. Aboral to the œsophagus there is nothing with which the cut edges of the body-wall can unite to give rise to a new mouth. If a cut passing nearly through the body is made aboral to the œsophagus then the inrolled cut margins aboral to the cut unite with the cut ends of mesenteries over a large part of the circumference and are held in the inrolled position long enough for the formation of a new mouth. As a matter of fact, however, in such cases the part of the body oral to the cut, being united with the aboral portion only by a slender strip of the body-wall; separates after a few days and the regenerating disc is no longer supplementary but terminal on the aboral piece and regenerates in the typical manner. Owing to this separation I have not yet succeeded in bringing about the production of supplementary discs with mouths in the region aboral to the œsophageal region.

As regards the relation between rapidity and amount of growth and the level at which these supplementary discs are situated, my own observations agree with those of Loeb. He found that the rapidity and amount of growth decreased with increasing distance from the oral end and that the extreme aboral region was incapable of producing supplementary discs. These results also agree with the observations described in my second paper (Child, '03*b*) concerning the effect of position upon rapidity and amount of regeneration.

General Considerations.

The formation of supplementary discs constitutes another chapter of evidence on the rôle of internal water-pressure in regulative processes in *Cerianthus.* It is scarcely necessary, I think, to devote further space to the consideration of Loeb's view that tentacle-reduction is due to loss of intracellular turgor. It certainly does not account for the observed facts to which Loeb attempts to apply it. A knowledge of the structure of the animal would have made such conclusions impossible.

These experiments show most clearly that continuous or nearly continuous tension due to internal pressure is absolutely essential for the persistence of form and structure in *Cerianthus.* In its absence atrophy always occurs.

Doubtless various changes in the intracellular turgor occur during reduction and atrophy but these must be regarded a result rather than a cause, for so long as the tissue is subjected to the tension caused by internal water-pressure these changes in turgor do not occur.

The fact that the changes in *Cerianthus* which Loeb regarded as change in turgor are not such does not of course detract in any way from the value of the osmotic hypothesis in general. It is important, however, that the effects of distension of internal cavities with water or other fluids should be distinguished from the effects of intracellular phenomena. The case in hand is simply an incorrect application of the osmotic hypothesis.

On page 57 Loeb ('91) makes the following statement regarding the supplementary disc formed in the middle region of the body: "Diese neugebildete Kopf, b. Fig. 2, scheint ganz auf dem Ektoderm zu sitzen." It would not have required an extended examination to show the incorrectness of this conclusion. These lateral discs are no more wholly ectodermal than the terminal discs of normal animals (see the figures of this paper).

Heteromorphic Tentacles.

Experiments.

In two cases among the hundreds of pieces examined the appearance of tentacles on the aboral end of a piece has been observed. Both cases occurred in a single series and were apparently due to closure of the pieces in a peculiar manner. Although numerous attempts to obtain additional cases were made, none were successful, the pieces failing to close in the proper manner. I have little doubt that whenever closure takes place in a certain manner to be described heteromorphic tentacles will be formed.

The pieces which afforded this peculiar result belonged to a series intended for the study of the possible methods of closure. The pieces were prepared as follows. A piece was cut oral to the middle of a specimen by two transverse cuts as indicated in Fig. 16. The oral end of the piece was in all cases just below the disc in the œsophageal region, the aboral end a considerable distance aboral to the aboral end of the œsophagus. The cylindrical piece thus obtained was divided longitudinally into halves as indicated by the small diagram to the right of Fig. 16. Each piece then represented half of the circumference of the body and œsophagus. The body-wall of the piece was bounded on all sides by cut surfaces, two transverse at the ends and two longitudinal at the sides. The portion of the œsophagus in the piece was bounded orally and laterally by cut surfaces, but aborally it terminated in the normal manner.

Pieces of this kind close in various ways. Some roll up longitudinally and form almost perfectly typical animals, others roll in part longitudinally and in part transversely and the cut surfaces of œsophagus and body-wall unite according to chance, *i. e.*, union of the adjacent cut surfaces occurs, whatever these may be. Many bizarre forms are produced, but they afford nothing new in principle. One important fact is shown by many pieces including the two which possess special interest at present, viz., that the aboral end of the œsophagus, although not a cut surface is capable of union with a cut surface. It is of interest in this connection to note that nowhere else in the body does the body-wall terminate in a free margin. This free margin behaves like a cut surface and may like any cut surface give rise to new tissue after union with another cut surface (see Child, '04*a*).

In cases where these pieces succeed in rolling up longitudinally so that the longitudinal cut surfaces of body-wall and those of the œsophagus unite, a more or less typical mouth and œsophagus result from the union and regeneration may proceed in an almost typical manner. Usually, however, the œsophagus unites with the body-wall as in œsophageal pieces (Child, '04*d*), either on all sides or partially. Aboral union between œsophagus and body-wall is made possible by the bending orally of the aboral part of the body-wall so that aboral end of the œsophagus and aboral cut surface of the body-wall unite. In all cases where union between œsophagus and body-wall occurs elsewhere than at the oral end a closed piece without a mouth is the result and a mouth cannot regenerate. Since there is no communication between enteron and exterior in such pieces it might be expected that they would behave as regards tentacle-regeneration like the œsophageal pieces (Child, '04*d*) *i. e.*, that they would become slightly distended at first and regenerate small tentacle-buds but would afterwards collapse and the tentacles would be reduced. As a matter of fact, however, these pieces though never approaching pieces which possess mouths often show a far greater power of tentacle-regeneration than œsophageal pieces. They frequently regenerate tentacles 5–6 mm. in length, *i. e.*, twice as long as those of the best œsophageal pieces. They may remain distended for a month or more but sooner or later collapse occurs and the tentacles undergo reduction. Since these pieces are completely closed like the œsophageal pieces they can become distended only as fluid passes through the body-wall ('04*d*). The important point is that the distended condition continues for a much longer time than in œsophageal pieces. The reason for this difference is probably to be found in the fact that pieces cut as in Fig. 16 contain not only the entoderm of the œsophageal region but the great mass of mesenterial differentiations aboral to the œsophagus, *i. e.*, the chief digestive region of the entoderm. There is little doubt that processes of secretion are much more active and persistent in this region than in the œsophageal entoderm, and consequently the quantity of fluid reaching the enteron either in connection with the secretory process or by osmosis through the body-wall in consequence of the presence of soluble substances secreted into the enteron is greater than in œsophageal pieces and distension continues for a longer time. Whatever the exact explanation may be the fact is patent in all cases. As will appear below, it is perhaps of considerable importance in connection with the appearance of heteromorphosis.

The pieces of this series were prepared September 26, 1902. All pieces were observed at intervals after the operation and among the methods of closure were found those represented diagrammatically in longitudinal section in Figs. 19 and 26. The body-wall and the œsophagus are indicated as in previous figures. In Fig. 26 the aboral end of the body-wall has bent over orally much farther than in Fig. 19. In both cases, however, the margin of the body-wall has united on all sides with that of the œsophagus, thus forming a closed piece composed partly of œsophagus and partly of body-wall. The enteric cavity of this piece is of course the enteric cavity of the lateral half of the body in the region from which it was taken. In Figs. 19 and 26 the external surface of the œsophageal portion on the right of the figure is ectodermal, but in the normal animal formed the axial surface of the œsophagus. The only essential difference between such pieces and œsophageal pieces (Child, '04*d*) consists in the greater length of the body-wall in the former.

Tentacle-regeneration at the oral ends of the pieces occurred in the typical manner (Figs. 20 and 27), each producing about half the number of tentacles possessed by the parent specimen, a result to be expected from the number of intermesenterial chambers contained in each piece.

About two months later (November 20) two of the pieces showed tentacles beginning to develop on the aboral ends. One of these pieces is shown in Fig. 17. The tentacles on the oral end had already attained their maximum development and were undergoing reduction, and in the case of the marginal tentacles, atrophy. On the aboral end of the piece four tentacle-buds appear. These seem to arise either from the œsophageal tissue or from the line of union between the aboral end of the œsophagus and the body-wall. The relations of parts is illustrated by the

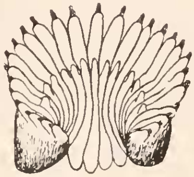
FIG. 17.

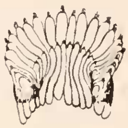
FIG. 18.


FIG. 19.


FIG. 20.

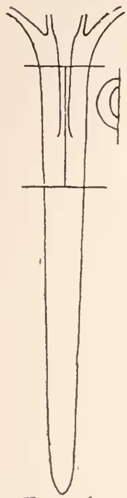
FIG. 16.


FIG. 21.


FIG. 22.


FIG. 23.

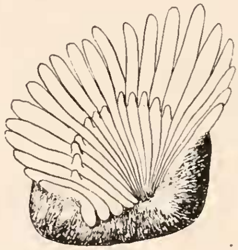
FIG. 24.


FIG. 25.

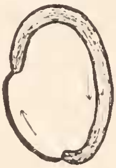
FIG. 26.


FIG. 27.


FIG. 28.

diagrammatic longitudinal sections, Figs. 19–22. Figs. 19–21 show the closure and the course of regeneration during the first two months. Fig. 22 represents a section of the stage shown in Fig. 19, and here the relation of the aboral tentacles and œsophagus and body-wall is seen. During the following months the number of aboral tentacles increased (Fig. 18, December 28) though the piece as a whole was undergoing reduction in size. Fig. 23 represents the section at this stage.

The other piece in which aboral tentacles developed is shown in Fig. 24 as it appeared two months after the operation (November 20). The method of closure in this piece differs somewhat from that occurring in the first: Figs. 26–28 illustrate this point. It will be seen that the aboral end of the body-wall bent orally much further than in the first piece (cf. Figs. 19 and 26), but also united with the aboral end of the œsophagus. It is probable that the piece was longer than the preceding. The difference in method of closure is readily accounted for by such difference in length. At the stage shown in Fig. 24 the aboral tentacles were beginning to develop. Fig. 28 is a diagrammatic section of this stage. Figure 25 represents a side view of the piece a month later (December 28). As the figure shows, the large tentacle in the middle arose just over the region where the body-wall folded longitudinally upon itself. (The shading of the body-wall represents the longitudinal striping.) All tentacles to the left of this large tentacle are upon the oral end of the piece, and all to the right are aboral. Six aboral tentacles appeared on this piece in addition to the single tentacle which can scarcely be regarded as either oral or aboral. In this case, as in the preceding, the aboral tentacles arose either from the œsophagus or from the line of union between it and the body-wall (Fig. 28).

Figs. 19–23 and 26–28 show that reduction of the body-wall is more rapid than reduction of the œsophageal region. This difference is probably correlated with functional differences in the tissues. Normally the body-wall is subjected to greater tension than the œsophagus, for the two sides of the latter are simply appressed during distension of the body. This being the case, it follows that conditions of tension depart less widely from the norm for the œsophagus than for the body-wall in these pieces, hence the more rapid change in the latter.

General Considerations.

These aboral outgrowths have been called tentacles for, in my opinion no ground exists for believing that they are anything else. It is evident from Figs. 17 and 24 that they arise in connection with intermesenterial chambers, for the lines extending aborally from the bases of the oral tentacles represent grooves marking the lines of attachment of the mesenteries to the œsophagus. Moreover, Fig. 24 shows that the aboral tentacles arise from those intermesenterial chambers which are least contracted for the only intermesenterial chambers which are at all distended at the aboral end are those from which the aboral outgrowths arise. In these respects these structures resemble the normal tentacles, and these facts lend color to the view that the conditions of origin of these aboral tentacles are similar to those of the normal tentacles.

In Figs. 19–23 and 26–28 the course of the intermesenterial circulatory currents is represented by arrows. These pass orally along the body-wall and aborally along the œsophagus. In both pieces the aboral end of the œsophagus bulges outward just oral to the line of union with the body-wall.

From Figs. 20 and 27 it is seen that this condition presents an obstruction in the course of the current in the aboral direction along the œsophagus, *i. e.*, local pressure upon the wall may occur. Except that they are reversed in position conditions here do not differ essentially from those described above leading to the formation of supplementary discs in the middle region (see Figs. 12–14). In brief, the suggestions regarding local pressure due to circulatory currents are as readily applicable to these cases as to the normal oral tentacles. Moreover, there is no adequate explanation on any other basis for the fact that these aboral tentacles arise from œsophageal tissue, or from the line of union between œsophagus and body-wall, rather than from the body-wall, like tentacles at the oral end. In my opinion the occurrence of these heteromorphic tentacles constitutes another important piece of evidence in support of the views already set forth (Child, '04*b*, '04*c*). The case of the single large tentacle which arose from the new tissue at the union of œsophagus and the folded body-wall (Fig. 25) is peculiar. The size of the tentacle is probably due to the greater distance between mesenteries, itself the result of stretching or growth of the new tissue at this point. The impossibility of ascertaining the exact internal condition in this case is evident. Opening of the piece must of course lead to extensive contraction, and fixation of these forms likewise causes extreme distortion as regards details, even after anesthesia. In external examination of the piece it could be seen, however, that a wide space between mesenteries existed here in the new tissue and from this region the large tentacle arose. What the course of the circulatory currents may have been it is also impossible to say. The parts which fused about this region were a part of the aboral end and a part of the longitudinal cut surface of the œsophagus, a part of the aboral end, the longitudinal cut surface and a part of the oral end of the body-wall. It is not impossible that both orally directed currents along the body-wall and aborally directed currents along the œsophagus may have been concerned. Moreover, regulative changes may have occurred in the distribution and direction of movement of the cilia. Thus, except for the great space between mesenteries, there is no definite evidence regarding the special factors concerned in the origin of this peculiar tentacle. Nevertheless, I think the conclusion is justifiable that the conditions of origin are not different in this case from those in cases already discussed. It is certain at any rate that some degree of distension is necessary for the formation of these aboral tentacles.

One important difference between the aboral tentacles and oral tentacles appears in both pieces, viz., the difference in time of appearance. In both cases the marginal tentacles at the oral end appeared within a few days after section, and the labial tentacles somewhat later, but the tentacles on the aboral end appeared only after about two months when the internal pressure was decreasing and the pieces were becoming reduced in size. This difference in the time of appearance directs attention to an important problem, viz., that of polarity so-called. Is the later appearance of the tentacles at the aboral end to be accounted for by a difference in physiological condition between the two ends such that under stimuli the oral end gives rise to tentacles in a much shorter time than the aboral end or is it possible that in consequence of the chance relations between mesenteries œsophagus and body-wall in the two cases cited the stimulus became effective only at a much later stage than at the oral end?

The data afforded by the two pieces do not permit a decision between these alternatives. It is probable that extensive modifications of the greatly thickened and differentiated mesenterial margins aboral to the œsophagus occurred before relations between the intermesenterial chambers and the aboral end of the piece approached those at the oral end. If this is the reason for the late appearance of the aboral tentacles it is unnecessary to assume that the tissues of the aboral end are less capable of forming tentacles under proper conditions than those of the oral end or in other words the difference in the time of appearance of oral and aboral tentacles does not indicate the existence of a physiological polarity except so far as the structure at the aboral end was originally different from that at the oral end.

On the other hand it may be that the aboral ends of such pieces are inherently less susceptible to tentacle-forming stimuli and that, therefore, action of the stimuli during a much longer time than at the oral end is necessary. If it shall be possible in the future to obtain sufficient material of this kind for a thorough histological examination of the alterations in the mesenteries at the aboral ends of pieces we shall be better able, perhaps, to decide the question.

The œsophageal pieces described in a previous paper (Child, '04*d*) present conditions somewhat similar to those in the two pieces with heteromorphic tentacles, in that the œsophagus unites orally and aborally with the body-wall forming a series of closed intermesenterial chambers within which the circulatory currents pass orally on the body-wall and aborally on the œsophagus. If these currents are factors in tentacle-formation and if they are equal in force in both directions it would seem that tentacles must appear on both oral and aboral ends of such pieces at the same time. Yet in no case has such a result been attained. The œsophageal pieces regenerate short tentacles on the oral end, but the internal pressure soon disappears and regeneration is replaced by reduction. In the two pieces described in the present section the internal pressure continues for a much longer time than in the œsophageal pieces. It appears from this that œsophageal pieces might produce aboral tentacles if the internal pressure continued for a sufficiently long time. But even if this should prove to be the case the question as to polarity or structural conditions as the cause of the difference of time in the appearance of tentacles at the two ends would remain to be decided. In the œsophageal pieces, however, the structure and relations of mesenteries at the two ends are essentially the same, no more modification being necessary at one end than at the other to produce typical structural conditions for tentacle-formation. Since this is the case the absence of tentacles on the aboral ends of œsophageal pieces seems at present to indicate that the difference between the two ends as regards tentacle-formation consists in something else than gross structural relations of parts, *i. e.*, that some kind of polarity exists.

The nature of organic polarity is at present exceedingly obscure, but it is well known that the polarity of pieces as regards regeneration is dependent upon the previous relations of the pieces as parts of a whole. In pieces of the lower forms, such as *Hydra*, other hydroids, *Planaria*, etc., the polarity may not be manifest in any distinct structural differentiation at the two ends, but merely in their functional condition. The piece, retaining more or less completely the functional capacities of the whole from which it was taken, uses, or attempts to use, its parts in a similar manner, *i. e.*, the piece, in continuing its functional life, attempts in some degree to use its aboral or posterior end as the aboral or posterior end of the whole is used, and the same is true of its anterior end. The functional stimuli affecting these parts are similar to, though perhaps less powerful than those in the whole. It is probable that the regeneration of the characteristic structures at the two ends is closely correlated with these stimuli. When we can alter the conditions so that the aboral or posterior end is affected by external stimuli which typically affect the oral or anterior end while the "internal polarity" remains the same, the result depends upon the relative value of external and internal conditions as formative factors. Frequently in such cases the regeneration is delayed; apparently because of the "confusion" of stimuli, *i. e.*, the original stimuli and those resulting from the atypical orientation. Finally one or the other dominates and the "polarity" either remains as it was originally, or is reversed. In more highly organized forms, where internal factors are much more affective than external, reversal of polarity is rarely or never possible.

I think it probable that there is a difference in functional condition between the two ends of the pieces of *Cerianthus*, in consequence of which the one end is functionally oral and the other aboral. But if the aboral end be subjected to conditions which typically affect only the oral end, and if these conditions continue for a sufficiently long time, the response takes place and structures typically oral in character appear on the aboral end. Whether the inherent polarity or the altered external conditions will dominate in any particular case can be determined only by experiment.

Summary.

1. Supplementary partial discs with a number of tentacles corresponding to that portion of the whole circumference which they represent can be produced from lateral transverse cuts in the body-wall of *Cerianthus*.

2. If the lateral cut is in the œsophageal region and is deep enough to involve the œsophagus the supplementary disc possesses a mouth because the cut surfaces of the body-wall unite with the cut surfaces of the œsophagus, thus forming a second opening into the œsophagus. If the cut is below the œsophageal region the supplementary disc possesses no mouth. In the extreme aboral region of the body the formation of supplementary discs does not occur.

3. When the lateral cut is made in the œsophageal region the permanent collapse and atrophy of the tentacles and region directly above the cut occurs, or if these have been removed their regeneration is retarded and atrophy occurs after a time. This collapse and atrophy is due, not to loss of intracellular turgor but to decrease in the internal water-pressure since the enteric cavity of the region above the cut is completely shut off from the general enteric cavity and from the exterior.

4. In two cases the formation of tentacles on the aboral end of a piece of certain form was observed. In both of these cases the conditions of internal pressure were apparently similar to those which exist at the oral end, but the less rapid regeneration of the aboral tentacles indicates either a difference in structural relations at the two ends or a difference in "polarity."

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University of Chicago,
December, 1903.

BIBLIOGRAPHY.

Child, C. M.

'03a Form-Regulation in Cerianthus. I. The Typical Course of Regeneration. Biol. Bull., Vol. V., No. 5, 1903.

'03b Form-Regulation in Cerianthus. II. The Effect of Position, Size and Other Factors on Regeneration. Biol. Bull., Vol. V., No. 6, Vol. VI., No. 1, 1903.

'04a Form-Regulation in Cerianthus. III. The Initiation of Regeneration. Biol. Bull., Vol. VI., No. 2, 1904.

'04b Form-Regulation in Cerianthus. IV. The Rôle of Water-Pressure in Regeneration. Biol. Bull., Vol. VI., No. 6, 1904.

'04c Form-Regulation in Cerianthus. V. The Rôle of Water-Pressure in Regeneration: Further Experiments. Biol. Bull., Vol. VII., No. 3, 1904.

'04d Form-Regulation in Cerianthus. VI. Certain Special Cases of Regulation and their Relation to Internal Pressure. Biol. Bull., Vol. VII., No. 4, 1904.

'04e Form-Regulation in Cerianthus. VII. Tentacle-Reduction and Other Experiments. Biol. Bull., Vol. VII., No. 6, 1904.

Loeb, J.

'91 Untersuchungen zur Physiologischen Morphologie der Thiere. I. Heteromorphose. Wurzburg, 1891.

'03 Zusammenstellung der Ergebnisse einiger Arbeiten uber die Dynamik des thierischen Wachsthums. Arch. F. Entwickelungsmechanik, Bd. XV., H. 4, 1903.